

DEVELOPMENT AND VALIDATION OF SIMPLE, RAPID AND SENSITIVE UV, HPLC AND HPTLC METHODS FOR THE ESTIMATION OF PIRFENIDONE IN TABLET DOSAGE FORM

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ABSTRACT

UV, HPLC and HPTLC methods were developed and validated for the quantitative determination of Pirfenidone, a novel antifibrotic agent used in idiopathic pulmonary fibrosis. Chromatography was carried out by isocratic technique on reversed phase Eclipse XDB-C₁₈ column (150 x 4.6 mm, 5 μ m) with mobile phase consisting of phosphate buff: acetonitrile (pH 3.5) 72:28 v/v at flow rate 1mL/min. TLC was carried out by stationary phase precoated Silica Gel 60 F₂₅₄ TLC Plate using mobile phase Toluene: Methanol, 8:2 v/v. The UV spectrophotometric determination was performed at 311nm using solvent methanol. The proposed methods were validated according to ICH Q2-(R1) guidelines. The linearity range for Pirfenidone was 5-70 μ g/mL for HPLC, 800-1600 ng/spot for HPTLC and 10-60 μ g/mL for UV method. These methods were accurate and precise with recoveries in the range of 98.2-102.32 and relative standard deviation < 2%. The developed methods were successfully applied for determination of Pirfenidone in tablets.

Keywords: UV, HPLC, HPTLC, Pirfenidone, anti-fibrotic agent.

INTRODUCTION

Pirfenidone is a new drug approved for the treatment of rare and generally incurable idiopathic pulmonary fibrosis (IPF). Patients with IPF suffer from shortness of breath, cough and hampered daily physical activities. Treatment for IPF consists of oxygen therapy, pulmonary rehabilitation and lung transplant. Pirfenidone is the only therapeutic agent available for the treatment of IPF [1, 2]. It was initially approved in Japan and Europe followed by India and USA in 2010 and 2014 respectively. Pirfenidone or 5-methyl-1-phenyl-2-(1H)-pyridone (fig. 1) is a small simple heterocyclic molecule with molecular mass of 185.22 and can be administered orally. Literature survey revealed that HPLC and LC-MS/MS methods are reported for determination of Pirfenidone from biological fluids such as plasma, serum and urine [3] but very few methods have been reported for the estimation of Pirfenidone in pharmaceutical dosage form [4]. Oral tablet formulation containing active Pirfenidone equivalent to 200 mg is available in the territorial markets of Japan, Taiwan, Korea and India whereas it is available in capsule formulation in the USA. Assay or monograph for Pirfenidone or its formulation is not available yet in any pharmacopoeia. Recently we have reported a validated stability-indicating high-performance thin-layer chromatographic method for the estimation of Pirfenidone in tablet formulations [5]. It was learned that recently one report has published HPLC method for determination of Pirfenidone in tablet formulations but this method lacked accuracy and hence we are reporting a better assay including UV and HPTLC along with HPLC method for routine quality control of Pirfenidone formulations [6-9].

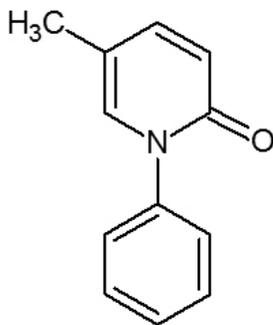


Figure 1. Structure of Pirfenidone.

EXPERIMENTAL

Chemicals and Materials

A request sample of Pirfenidone was received from Cipla India Ltd., Mumbai. Methanol AR, acetonitrile HPLC and water HPLC, toluene (AR) grade was purchased from Merck India limited. Film coated tablets of Pirfenidone (Pirfenex, cipla Ltd., Mumbai India) were procured from local market.

Instrumentation

The chromatographic system comprised of an Agilent 1200 series HPLC system equipped with quaternary gradient pump G1311A with On Line Degasser G1322A. Pre-filtered sample were injected in to Eclipse XDB C₁₈ column (150 x 4.6 mm, 5 μ m) using Rheodyne syringe loading sample injector (Rheodyne, Cotati, CA). The mobile phase consisted of phosphate buffer-acetonitrile (pH 3.5) (72:28%v/v). HPTLC instrument consists of CAMAG Linomat-IV TLC applicator, CAMAG TLC-Scanner-III (version 4.0.1) supported with CAMAG Wincats[®] software (version 4.0.1), CAMAG twin trough glass chamber (10 cm x 10 cm) for TLC development and Hamilton Syringe (100 μ L). The mobile phase consisted of toluene- methanol (8:2 v/v). A spectrophotometric system consists of a double beam Shimadzu UV/VIS spectrophotometer, UV-1700 having two matched 10 mm quartz cells with 1cm light path equipped with Shimadzu UV probe software was employed in spectrophotometric analysis.

Preparation of standard solution

To prepare standard solution, accurately weighed 100 mg of Pirfenidone was transferred in to 100 mL volumetric flask, dissolved and diluted up to the mark with methanol to obtain stock solution containing 1.0 mg/mL of the drug.

Preparation of sample solution

The sample solution was prepared by following method; twenty tablets containing Pirfenidone were accurately weighed and finely powdered. Accurately weighed amount of powder equivalent to 100 mg of Pirfenidone was transferred to 100 mL volumetric flask and 50 mL of methanol was added. The mixture was sonicated for 30 min, diluted to mark with methanol and filtered through whatman filter paper no.41 from the stock solution 1.0 mL was accurately transferred to 10 mL volumetric flask and diluted with methanol to obtain an intermediate solution of 100 μ g/mL of Pirfenidone. Aliquot of 1.0 mL of this solution was accurately diluted to 10 mL with diluents to obtain the final sample solution containing 10 μ g/mL of Pirfenidone. This was filtered through 0.45 μ m nylon filter, degassed by sonication and volume of 20 μ L was injected in HPLC system. As that same in high performance thin layer chromatography from the stock solution 1.0 mL was accurately transferred to 10 mL volumetric flask and diluted with methanol to obtain an intermediate solution of 100 μ g/mL of Pirfenidone was applied on HPTLC plate. In UV spectrophotometric sample preparation was same as that of HPTLC from the stock solution 1.0 mL was accurately transferred to 10 mL volumetric flask and diluted with methanol to obtain an intermediate solution of 100 μ g/mL of Pirfenidone. Aliquot of 1.0 mL

of this solution was accurately diluted to 10 mL with diluents to obtain the final sample solution containing 10 µg/mL of Pirfenidone.

Development of Spectrophotometric and Chromatographic methods

The UV absorption spectrum of Pirfenidone in methanol depicted λ_{\max} at 311 nm and a shoulder peak at 224 nm (fig.2A). Wavelength showing the maximum absorbance 311 nm was selected for measurement of absorbance. The HPLC conditions for Pirfenidone was optimal with isocratic mode Phosphate buff: acetonitrile (pH 3.5) 72:28 v/v at flow rate 1 mL/min in a total run time of ten minutes and was used to check the system suitability study. Fig.2B exhibits the corresponding chromatogram of standard Pirfenidone in optimized HPLC conditions showing retardation time for Pirfenidone at 5.56 min. HPTLC was performed on aluminum-backed Silica Gel 60 GF₂₅₄ HPTLC plate from

Merck, India. Sample solution was applied as 6 mm band and 4 mm spacing at a constant application rate of 5 sec μl^{-1} by means of Linomat IV sample applicator fitted with a 100- μl syringe. The Linear ascending development was performed in a CAMAG twin trough glass chamber with mobile phase consisting of toluene: methanol (8:2 v/v). The optimum time for saturation of the chamber with mobile phase vapor was 15 min at ambient temperature (25 ± 2 °C) and relative humidity $60 \pm 5\%$. The plates were allowed to develop at distance of 7.0 cm. Subsequent to development; the plates were dried in current of dry air by using compact hot air dryer. Plates were evaluated by densitometry at 315 nm by means of a CAMAG TLC scanner III with Wincat software version 4.0.1. Figure 2C displays the corresponding chromatogram of standard Pirfenidone in optimized HPTLC condition.

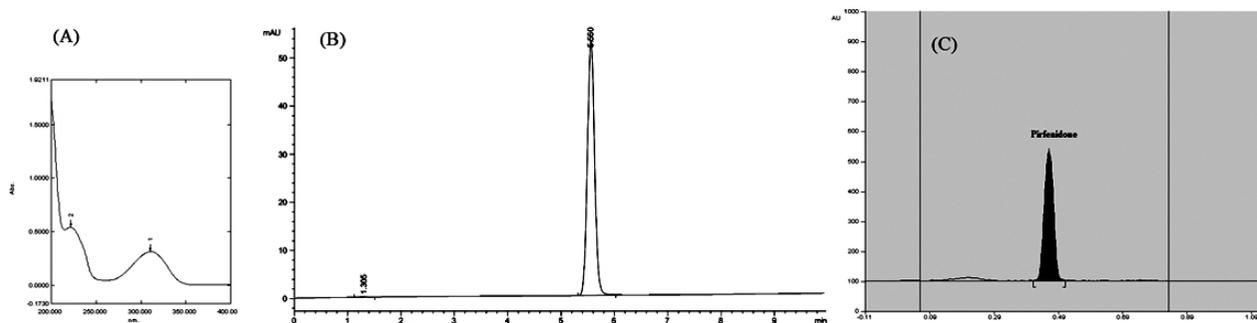


Figure 2. (A) UV spectra of Pirfenidone, (B) Chromatogram of Pirfenidone, (C) Densitogram of Pirfenidone.

Table 1. Validation parameters for determination of Pirfenidone by proposed UV, HPLC and HPTLC methods.

Validation of developed methods	
Parameters	UV Spectrophotometric method
Linearity range	10-60 µg/mL
Regression equation	$y = 0.039x$
Correlation coefficient	0.997
Intra- day precision (%RSD, n=3)	1.02
Inter-day precision (%RSD, n=3)	1.14
Reproducibility	1.79
Parameters	HPLC method
Retardation time \pm SD (min)	5.562
Capacity factor	2.724
Tailing factor	1.10
Theoretical plate	8511
Injection repeatability (%RSD n=5)	0.32
Linearity range (µg/mL)	5-70 µg/mL
Regression equation ($y=mx+c$)	$y=43.98x$
Correlation coefficient	0.9964
Intra-day precision (%RSD n=3)	0.3701
Inter-day precision (%RSD n=3)	0.6751
Reproducibility (%RSD, with different analyst)	2.12
Parameters	HPTLC method
Regression equation	$Y=7223.584+6.598$

Correlation coefficient	0.9954
Linearity	800-1600 ng/band
Precision (intraday) % RSD	1.42
Precision (interday) %RSD	1.68
LOD	0.550
LOQ	1.667
Specificity	Specific
Robustness	Method is robust
% Assay	99.12 \pm 1.28%
R_f	0.49 \pm 0.03

SD=standard deviation, RSD=relative standard deviation, y=concentration of Pirfenidone in µg/mL, x=peak area of Pirfenidone.

Table 2. Recovery studies for UV, HPLC and HPTLC method.

Recovery Studies			
Method	Amount taken (µg/mL)	Amount added (mg)	%Recovery (mean \pm SD)
UV	10	3.2	99.45 \pm 1.10
		7.5	98.20 \pm 0.75
		11	102.2 \pm 1.62
HPLC	10	3.2	101.32 \pm 1.25
		7.5	99.15 \pm 0.56
		11	99.28 \pm 1.32
HPTLC	100	3.2	102.32 \pm 0.55
		7.5	100.12 \pm 0.42
		11	99.98 \pm 1.44

Validation of the methods

The proposed methods were validated according to International Conference on Harmonization ICH Q2 (R1) guidelines. The method was

validated using various parameters such as precision, accuracy, specificity and linearity [9-17]. The system suitability and validation parameters for determination of Pirfenidone by HPLC method are depicted in table 1. Linear correlation was found between peak areas versus concentration of Pirfenidone in the range of 5-70 µg/mL the result of injection repeatability (RSD<1%), intraday and interday precision (RSD<1%) and method reproducibility (RSD<2%) proved the method to be precise. The % recovery was found to be in the range of 99.15-101.32% (table 2). The limit of detection (LOD) and limit of quantification (LOQ) was found to be 0.04 and 0.14 µg/mL of Pirfenidone respectively. The specificity of the method was assessed by comparing chromatograms of Pirfenidone obtained from standard solution of drug and that obtained from formulation sample solutions (fig 2). The retardation time of the drug from standard and sample solution was identical. The comparison of chromatograms confirmed that excipients did not interfere in the separation of Pirfenidone. Peak purity check of Pirfenidone peaks obtained from standard solution was found to be 0.9964, conforming to the specificity of the method. This was further supported by good correlation ($r=0.996$) between the PDA spectrum of Pirfenidone from standard solution and that from sample solution of formulation. The change (% 5) in proportion of organic solvent, the change ± 2 in wavelength and flow rate did not show significant variation in the area for Pirfenidone (%RSD<2). The stability of working standard solution of Pirfenidone was evaluated to verify that any spontaneous degradation occur when the sample were prepared. The stability profile for sample solution of Pirfenidone was studied at different conditions such as normal, acidic, basic, oxide, heat and sunlight. The results were expressed as percentage of drug remaining. The data obtained showed that sample solution was stable during all the conditions with degradation less than 2% and hence the graphs are not presented.

The validation parameters for determination of Pirfenidone by HPTLC method are depicted in table 1. Linear correlation was found to be in the range of 800-1600ng/spot respectively. The result of repeatability (RSD<1%), intraday and interday precision (RSD<2%) and method reproducibility (RSD<2%) proved the method to be precise. The % recovery was found to be in the range of 99.28-102.32% (table 2). The specificity of the method was assessed by comparing chromatograms of Pirfenidone obtained from standard solution of drug and that obtained from formulation sample solutions (fig 2C). The R_f value of the drug from standard solution and from sample solution were identical. The comparison of chromatograms confirmed that excipients did not interfere in the separation of Pirfenidone. This was further supported by good correlation ($r=0.9954$). The change (%5) in proportion of mobile phase and change ± 2 in wavelength did not show significant variation in the area for Pirfenidone (%RSD<2). The stability of working standard solution of Pirfenidone was evaluated to verify that any spontaneous degradation occur when the sample were prepared. The stability profile for sample solution of Pirfenidone was studied at different conditions such as normal, acidic, basic, oxide, heat and sunlight. The results were expressed as percentage of drug remaining. The data obtained showed that sample solution were stable during all the conditions with degradation less than 2%. Validation parameters for determination of Pirfenidone by UV method are depicted in table 1. The Beer's range was found to be in the range of 10-60 µg/mL of Pirfenidone. The result of regression analysis and validation for determination of Pirfenidone by UV method are shown in table 1. The variability in the precision study was found within limits proving the method to be precise. The % recovery in accuracy study was found to be 98.20-102.2% (table 2). The limit of detection (LOD) and (LOQ) was found to be 0.08 and 0.24, respectively. The working solution of Pirfenidone in methanol was found to be stable for at least five days.

Tablet powder was sonicated with methanol for 30 min to ensure complete dissolution of drug. The developed UV, HPLC and HPTLC methods were applied to estimate content of Pirfenidone in marketed tablet formulation by our method. The amount of Pirfenidone was calculated using the respective regression equation obtained from calibration graph of UV, HPLC and HPTLC methods. Result of analysis of pharmaceutical formulation containing Pirfenidone is shown in table 3.

RESULT AND DISCUSSION

A single report is available on analytical method for determination of Pirfenidone in tablet/capsule formulation with UV and HPLC method [4], other available methods are from the Stability and pharmacokinetic point of view and needed very tedious process such as liquid-liquid extraction and hence are time consuming and non-economical [5,10]. The earlier reported HPLC method lacks accuracy with respect to peak sharpness and a single method

is reported. We have successfully utilized an isocratic elution technique at room temperature with UV detection for the determination of Pirfenidone in marketed formulation. This method is void of tedious sample preparation procedure and is highly economic. The real advantage of the proposed HPLC method is low retardation time of 5.56 min for Pirfenidone, this time is higher than the earlier report but presents sharp result. The real advantages of the proposed HPTLC method are high sample throughput with small amount of solvent requirement and simple sample preparation techniques.

Table 3. % Estimation of Pirfenidone formulation

Dosage form	Labeled claim (mg)	Methods	% Content of drug \pm SD
Tablet	200	UV	101.23 \pm 1.65
		HPLC	99.47 \pm 1.34
		HPTLC	99.23 \pm 0.77

CONCLUSION

To conclude with, reported UV, HPLC and HPTLC method for the determination of Pirfenidone in pharmaceutical formulation were found to be highly economical, simple, sensitive, accurate, precise and rapid. Moreover the HPTLC method presented was found to be rather simple and time saving alternative to HPLC method of analysis for Pirfenidone for routine quality control of pharmaceutical formulation.

ACKNOWLEDGMENT

Authors acknowledge the Cipla Pharmaceuticals Pvt. Ltd for gift sample of Pirfenidone.

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